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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

1941

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A COMPARISON OF THE OFFICIAL AND THE CHICKEN METHODS FOR THE OXYTOCIC BIOASSAY OF POSTERIOR PITUITARY PREPARATIONS[1,2]

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The purpose of this investigation was to compare the chicken blood pressure and the official uterine methods for the oxytocic bioassay of posterior pituitary preparations.

The present official method (1) is essentially that described by Dale and Laidlaw (2) in 1912. It has not been uniformly successful in the hands of all bioassayists and has been criticized by many workers from Hamilton and Rowe (3) in 1916 to Morrell, Allmark, and Bachinski (4) in 1940.

The chicken blood pressure depressor method was introduced in 1939 by Coon (5), who claimed many advantages for it. This method is based on the fact, first described by Paton and Watson (6) in 1912, that the intravenous injection of posterior pituitary solution elicits a precipitous and fleeting fall of blood pressure in the bird. This response was shown by the same workers to be accompanied by a splanchnic vasodilatation. Hogben and Schlapp (7) showed that this effect was not due to histamine or histamine-like substances, and Hogben (8) proved the ability to elicit the avian depressor response to be a specific property of posterior pituitary extracts. Following the separation of the pressor and oxytocic principles by Kamm and his associates (9), Gaddum (10) and subsequently Morash and Gibbs (11), Holtz (12), and Dietel (13), found the response to be produced by smaller doses of pitocin than of pitressin. This observation was used by Geiling (14) and by Sealock and duVigneaud (15) for estimating oxytocic activity, but Coon was the first to determine the conditions for its use as the basis for a reliable method of bioassay.

The question naturally arises as to the agreement of the two methods. Coon found that they agree for preparations such as solution of posterior pituitary and pitocin in which the pressor-oxytocic ratio is one or less. He also found, however, that the results of the two methods disagree for preparations which have a pressor-oxytocic ratio greater than 2.5:1. In such cases the discrepancy may reach 400% increasing with the pressor-oxytocic ratio. As Coon pointed out, there was no evidence to indicate which of the methods was at fault.

In view of the advantages claimed for the chicken method, it appeared worth while to investigate the technic thoroughly in order to discover the factors responsible for the discrepancy in results noted above.

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

[2] A report covering part of this work was presented before the American Society for Pharmacology and Experimental Therapeutics on April 19, 1941.

Experimental. Pituitary preparations having pressor-oxytocic ratios ranging from 1:15 to 30:1 were assayed by both the official and the chicken methods for oxytocic activity. The two methods agreed for preparations having pressor-oxytocic ratios ranging from 1:15 to 3.5:1. With preparations having ratios ranging from 3.5:1 to 30:1 the chicken method gave higher results; the discrepancy varied with the pressor-oxytocic ratio (fig. 1) and reached 350% in the case of pitressin, which had a ratio of 30:1. These figures differ slightly from those of Coon but sustain his conclusion that "the pressor component must exceed the oxytocic component by more than 250% before it will introduce significant discrepancy in assay results by the two methods." The results admit of two possible explanations. Either the presence of relatively large amounts of pressor principle augments the depressor response, causing the chicken method to give high results; or such a preponderance of pressor principle antagonizes the

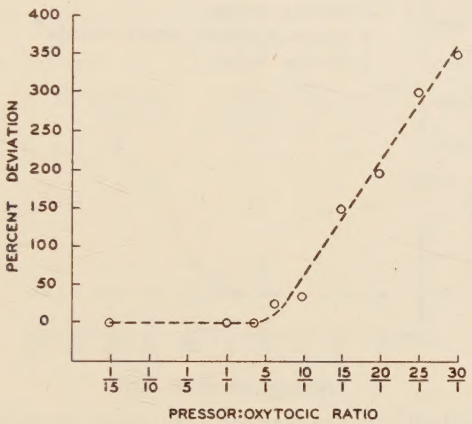

Fig. 1. Curve Expressing the Relation between the Pressor-Oxytocic Ratio of the Preparations and the Percentage Deviation of the Chicken Assay Values from Those Obtained by Means of the Official Method

action of the oxytocic principle on the isolated guinea pig uterus, causing the uterine method to give low assay values.

In order to discover which method was at fault the following experiment was carried out. Samples of pitressin and pitocin[3] were assayed by both methods for oxytocic activity, and for pressor activity on the anesthetized cat. From these two preparations mixtures were prepared having known oxytocic and pressor potencies and, therefore, known pressor-oxytocic ratios, the latter ranging from 1:15 for pitocin to 30:1 for pitressin. These mixtures were then assayed for oxytocic activity by both methods. The results are shown in fig. 2. The increase of the pressor-oxytocic ratio of pitocin due to admixture with pitressin in varying proportions did not alter the oxytocic values of the mixtures as determined by the official uterine method. Therefore it is apparent that the presence

[3] The author wishes to express his appreciation to Dr. Oliver Kamm of Parke, Davis and Company for supplying these materials.

of the pressor principle in preponderant amounts does not inhibit the action of the oxytocic principle on the isolated uterus. It appears, then, that the presence of relatively large quantities of pressor principle in preparations having high pressor-oxytocic ratios augments the avian depressor response to the oxytocic component of such preparations.

In 1939, Fraser (16) reported that postlobin-V (pressor) appeared four times as active when compared with postlobin-O (oxytocic) by the uterine method, using the Van Dyke-Hastings modification of Tyrode's solution, as it did when compared with the same standard by the same method using Locke's solution. He found that the high magnesium concentration of the Van Dyke-Hastings solution, 2.5 mgm. %, was the factor responsible for the discrepancy, and that when the magnesium was replaced with its osmotic equivalent of sodium chloride

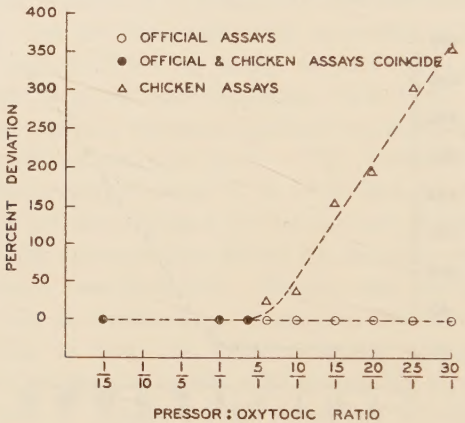

FIG. 2. CURVE ILLUSTRATING THE PERCENTAGE DEVIATION OF OFFICIAL AND CHICKEN ASSAY VALUES OF SOLUTIONS WITH VARYING PRESSOR-OXYTOCIC RATIOS FROM THE VALUES CALCULATED FROM THE OFFICIAL POTENCIES OF THE PITOCIN AND PITRESSIN FROM WHICH THEY WERE PREPARED

the assay values corresponded with those obtained when Locke's solution was used.

The official Locke-Ringer solution contains only a trace of magnesium, and it appeared possible, in the light of Fraser's findings, that the magnesium concentration of chicken blood serum might be responsible for the observed high oxytocic values of pressor preparations when assayed by the chicken method. According to Eveleth (17), the average serum magnesium level of chickens is 2.8 mgm. %. In order to test this hypothesis, solutions with varying pressor-oxytocic ratios which had already been assayed by the official and chicken methods were reassayed by the uterine method, using the official Locke-Ringer solution with the magnesium concentration adjusted to 2.5 mgm. %. The results (table 1) lend support to the hypothesis. Preparations having pressor-oxytocic ratios ranging from 1:15 to 3.5:1 exhibited the same oxytocic potencies by all three methods. When the ratio exceeded 3.5:1 the chicken method and

the uterine method using a magnesium concentration of 2.5 mgm. % agreed, both giving higher values than the official method. This experiment confirms the conclusion of Fraser that "the degree of oxytocic activity observed in an assay depends on the magnesium concentration of the uterine bath, when the ratio between oxytocic and pressor activities of the standard preparation is different from that of the unknown preparation." The experiment shows further, however, that the magnesium concentration of the bath does not affect assay results unless the pressor-oxytocic ratio exceeds 3.5:1.

In order to adduce further evidence that the serum magnesium level was the factor responsible for the high chicken assay values, an attempt was made to potentiate the depressor response to pituitary preparations by the intravenous injection of magnesium immediately preceding the injection of the pituitary.

TABLE 1

Comparison of results obtained by the official, chicken, and high magnesium uterine methods of oxytocic assay

PITUITARY SOLUTIONS	PRESSOR OXYTOCIC RATIO	CALCULATED VALUES* PER CENT U. S. P. STANDARD	OFFICIAL METHOD PER CENT U. S. P. STANDARD	CHICKEN METHOD PER CENT U. S. P. STANDARD	HIGH Mg UTERINE METHOD PER CENT U. S. P. STANDARD
Pitocin	1:15		11,000	11,000	11,000
U. S. P. posterior pituitary	1:1		100	100	100
High P:O 1	3.5:1	57	57	56	53
High P:O 2	6.3:1	64	64	80	80
High P:O 3	9.9:1	75	75	100	100
High P:O 4	15:1	100	100	250	250
High P:O 5	20:1	75	75	222	208
High P:O 6	25:1	60	60	240	216
Pitressin	30:1		150	675	675

* Calculated on the basis of the official assay values of the pitocin and pitressin from which these solutions were prepared.

Such an experiment is shown in fig. 3. An injection of pitressin (PR) was made. Several minutes later 2.0 cc. of 0.9 % sodium chloride solution (PSS) were injected as a control; this was followed immediately by the same dose of pitressin as was given previously. The pitressin elicited the same depressor response as before. After the blood pressure had returned to normal, 2.0 cc. of an approximately isotonic magnesium chloride solution, containing 5 mgm. of magnesium, were injected and the same dose of pitressin repeated. The depressor response was then much greater than with pitressin alone. The second tracing in fig. 3 illustrates the same fact. The potentiating effect of the magnesium disappeared after 20 to 25 minutes; serum magnesium determinations showed the level to be only slightly elevated after 20 minutes had elapsed and to be approximately normal 25 to 30 minutes after the injection. In order to estimate the magnitude of this potentiating effect, the experiment illustrated in fig. 4 was carried out. An injection of pitressin (PR) caused a blood pressure

fall of 40 mm. However, following the intravenous injection of 5.0 mgm. of magnesium one half the previous dose of pitressin elicited the same fall of blood pressure. Thus the magnesium increased the sensitivity to the pitressin by 100%. Similar experiments showed that the avian depressor responses to U.S.P. standard posterior pituitary solution and pitocin were not significantly affected by the injection of magnesium. This latter finding is what would be expected in view of the report of Fraser that 10 mgm. % anhydrous magnesium chloride (approximately 2.5 mgm. % magnesium), a value which closely approaches the

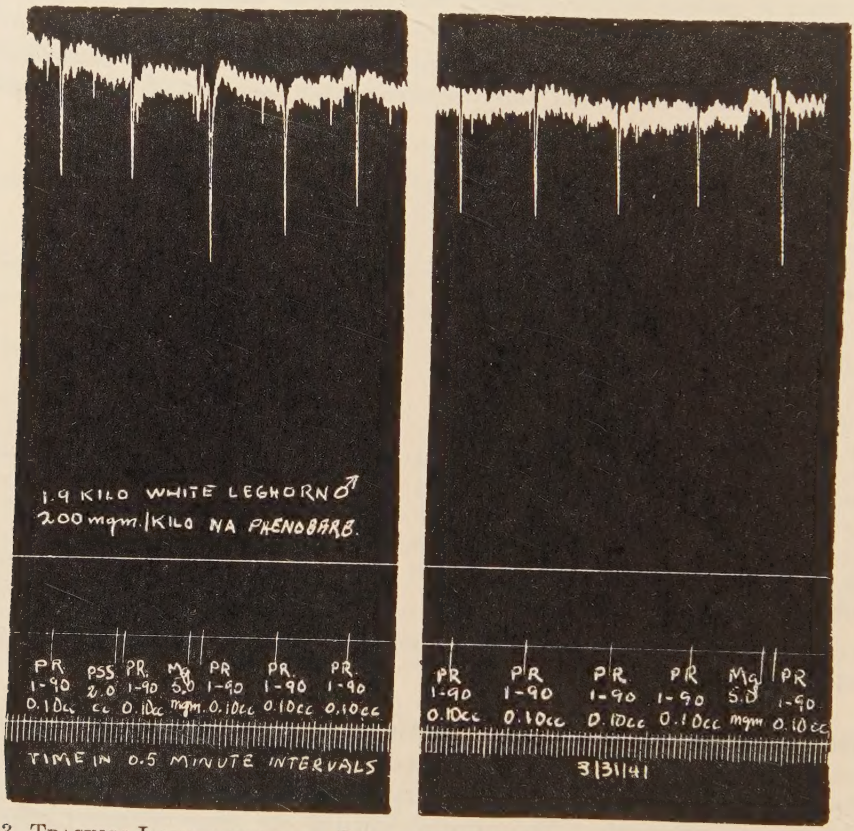

FIG. 3. TRACINGS ILLUSTRATING THE POTENTIATION OF THE AVIAN DEPRESSOR RESPONSE TO PITUITARY PREPARATIONS BY THE INTRAVENOUS INJECTION OF MAGNESIUM

normal serum magnesium level of the chicken, is the level at which postlobin-O exerts its maximum effect on the isolated guinea pig uterus.

In a few preliminary experiments the effect of the intravenous injection of potassium, sodium, calcium, zinc, and cadmium compounds on the sensitivity of the depressor response to subsequent injections of pituitary preparations was studied. None of the ions exerted any appreciable influence on the response.

It was noted by Coon that when a series of small doses of pituitary are injected over a period of time, the depressor sensitivity may decrease to such an

extent that five to eight times the original dose must be injected in order to elicit a response equal to the original one. Similarly, the administration of 25 units of pitocin in one dose will abolish the depressor response to subsequent smaller doses. Since an increase in serum magnesium increases the sensitivity, it was thought possible that the decrease in sensitivity after large doses of pituitary preparations might be due to a fall in the level of the serum magnesium. An experiment was carried out in which 0.2 cc. of a 1–10 dilution of U.S.P. standard solution was injected at three to five minute intervals until the dose no

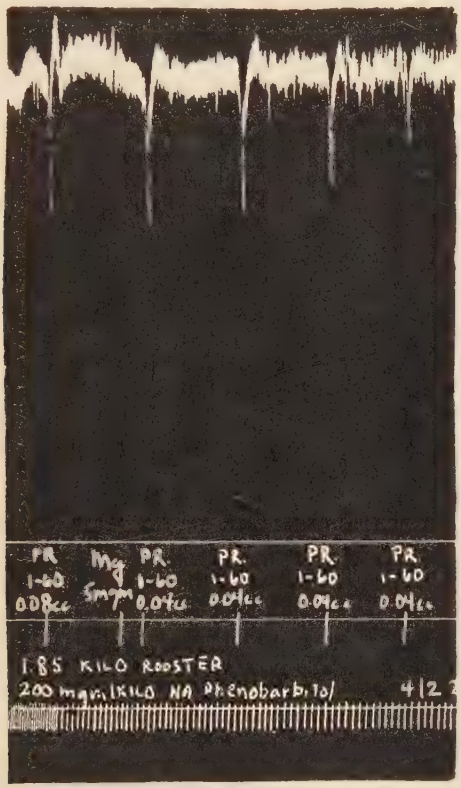

FIG. 4. TRACING ILLUSTRATING A 100 PER CENT POTENTIATION OF THE AVIAN DEPRESSOR RESPONSE TO PITRESSIN BY THE INTRAVENOUS INJECTION OF MAGNESIUM

longer elicited any response. Serum magnesium determinations showed that the loss of sensitivity was not accompanied by any significant variation of the serum magnesium level. Similarly, a massive dose of pitocin (25 U.S.P. units), which abolished the response to subsequent smaller doses, did not change the serum magnesium.

DISCUSSION. The chief disadvantage of the chicken method is the fact that it gives higher values for preparations with high pressor-oxytocic ratios than the official method. This discrepancy is shown to be related to the fact that the serum magnesium level of the chicken is higher than the magnesium titer of the

official Locke-Ringer solution, which contains only a trace of this ion. This conclusion is justified because when the magnesium concentration of the official Locke-Ringer solution is raised to 2.5 mgm. %, the approximate serum magnesium level of the chicken, the uterine and chicken methods give oxytocic values which agree for the various types of posterior pituitary preparations studied, including those with high pressor-oxytocic ratios. The question now arises as to whether the assays obtained for preparations with high pressor-oxytocic ratios by means of the official method are valid, or whether the higher values, obtained by the chicken method and the uterine method using Locke-Ringer solution with 2.5 mgm. % magnesium, are the more nearly correct. Such preparations are not used for their oxytocic activity, but if any specific oxytocic activity is stated to be present it should be that which the preparation will probably exert when used in man. It was demonstrated by Fraser and and confirmed in the present investigation that the action of preparations with high pressor-oxytocic ratios on the isolated uterus of the guinea pig depends on the concentration of magnesium in the medium bathing the muscle. That this relationship also holds true for the vascular musculature of the chicken *in situ* is shown by the fact that raising the serum magnesium level increases the sensitivity of the depressor response to subsequent doses of high pressor-oxytocic preparations. The serum magnesium level of man is 2.7 mgm. $\pm$0.3 mgm. %, according to Greenberg and his associates (18). It appears probable, therefore, that when preparations with high pressor-oxytocic ratios are used in man they exert an oxytocic activity which approaches more closely the potency indicated by the chicken method and the uterine method using high magnesium Locke-Ringer solution than that indicated by the official method.

As reported by Coon and verified in the present investigation, the chicken method possesses many practical advantages over the official uterine assay. These advantages include relative simplicity and economy of time and funds. It is felt in this laboratory that this new method should receive consideration for official recognition.

SUMMARY

1. The finding of Coon that the chicken method yields higher oxytocic values than the official method for preparations which have high pressor-oxytocic ratios is confirmed.

2. This discrepancy is explained by the difference between the low magnesium concentration of the official Locke-Ringer solution and the higher magnesium concentration of chicken blood serum. When the magnesium titer of the Locke-Ringer solution used in the official uterine method is raised to 2.5 mgm. %, the approximate serum magnesium level of the chicken, the discrepancy disappears.

3. The conclusion of Fraser that "the degree of oxytocic activity observed in an assay depends on the magnesium concentration of the uterine bath, when the ratio between the oxytocic and pressor activities of the standard preparation is different from that of the unknown preparation" is confirmed and extended.

4. Raising the serum magnesium level of the chicken by the intravenous in-

jection of magnesium chloride increases the sensitivity of the depressor response to preparations having high pressor-oxytocic ratios but not to preparations having a ratio of one or less. The effect wears off rapidly, disappearing entirely at approximately the same time that the serum magnesium level returns to normal.

5. The oxytocic activity which a preparation with a high pressor-oxytocic ratio will exert when used in man is probably indicated more accurately by the chicken method and the uterine method, using a Locke-Ringer solution with a magnesium concentration of 2.5 mgm. %, than by the official uterine method.

6. Neither the progressive decrease in sensitivity of the avian depressor response, which accompanies the injection of a series of small doses of posterior pituitary preparations, nor the abolition of the response after a massive dose of pitocin is due to a fall in the blood serum magnesium level.

7. The claims of Coon as to the many practical advantages of the chicken method are confirmed. The technique should receive consideration for recognition as an official method of assay.

The author wishes to express his appreciation to Doctors E. M. K. Geiling and J. M. Coon for their criticisms and advice. He also wishes to thank Miss Elizabeth Webster for her assistance. Acknowledgment is made of grants from the Board of Trustees of the United States Pharmacopoeia and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago, which aided the investigation.

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